Polynucleotide kinase and aprataxin-like forkhead-associated protein (PALF) acts as both a single-stranded DNA endonuclease and a single-stranded DNA 3' exonuclease and can participate in DNA end joining in a biochemical system.

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Authors: Sicong Li, Shin-ichiro Kanno, Reiko Watanabe, Hideaki Ogiwara, Takashi Kohno, Go Watanabe, Akira Yasui, Michael R Lieber

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Public Summary:
All human DNA repair processes require a nuclease to remove damaged DNA. Metaphorically speaking, a nuclease is like a surgical scalpel that removes the damaged tissue, but in this case the damage is at the molecular level in the DNA. NHEJ is the name of one of the five major DNA repair types. We had previously identified the major nuclease for NHEJ. In this paper, we identify a secondary nuclease for this pathway. The significance of this for stem cell gene correction is as follows: if we could block the NHEJ pathway, this would improve gene targeting by directing the correction process along the pathway (homologous recombination) that achieves the gene correction. By identifying and understanding all of the components of the relevant repair pathways, we have a more intelligent way of improving gene targeting efficiency

Scientific Abstract:
Polynucleotide kinase and aprataxin-like forkhead-associated protein (PALF, also called aprataxin- and PNK-like factor (APLF)) has been shown to have nuclease activity and to use its forkhead-associated domain to bind to x-ray repair complementing defective repair in Chinese hamster cells 4 (XRCC4). Because XRCC4 is a key component of the ligase IV complex that is central to the nonhomologous DNA end joining (NHEJ) pathway, this raises the possibility that PALF might play a role in NHEJ. For this reason, we further studied the nucleolytic properties of PALF, and we searched for any modulation of PALF by NHEJ components. We verified that PALF has 3' exonuclease activity. However, PALF also possesses single-stranded DNA endonuclease activity. This single-stranded DNA endonuclease activity can act at all single-stranded sites except those within four nucleotides 3' of a double-stranded DNA junction, suggesting that PALF minimally requires approximately four nucleotides of single-strandedness. Ku, DNA-dependent protein kinase catalytic subunit, and XRCC4-DNA ligase IV do not modulate PALF nuclease activity on single-stranded DNA or overhangs of duplex substrates. PALF does not open DNA hairpins. However, in a reconstituted end joining assay that includes Ku, XRCC4-DNA ligase IV, and PALF, PALF is able to resect 3' overhanging nucleotides and permit XRCC4-DNA ligase IV to complete the joining process in a manner that is as efficient as Artemis. Reduction of PALF in vivo reduces the joining of incompatible DNA ends. Hence, PALF can function in concert with other NHEJ proteins.